Steven D. Aust
Department of Biochemistry
Michigan State University
East Lansing, MI 48824-1319

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DEVELOPMENT OF THYROID HYPERPLASIA IN GREAT LAKES FISH RESULTING FROM CONTAMINATION BY DIOXINS AND RELATED CHEMICALS

Steven D. Aust
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1. Results and Conclusions:

A relatively high incidence of goiters has been reported in Great Lakes sport fish, with the incidence apparently correlated with the general level of contamination of the lake itself. The thyroid hyperplasia (goiter) appears unrelated to an iodine deficiency. We have suggested that the appearance of these goiters could be a function of contamination of the fish with dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD) or similarly-acting compounds, which are reported to have a significant goiterigenic activity, and a profound effect on thyroid hormone and vitamin A levels in the blood. We proposed therefore, to attempt to evaluate the correlation between thyroid hyperplasia and TCDD type contamination in Great Lakes sport fish. Specifically, the objective of the study was to determine the correlation between the incidence of thyroid hyperplasia, levels of thyroid hormones and vitamin A in the blood and the contamination of the particular fish by dioxin, or dioxin like contaminants, as measured by both analytical and bioassay methodologies.

Fifty-seven fish of three common sport species (Lake Trout, Coho and King Salmon) were sampled during the course of the study. Each fish was carefully examined for the presence of a growth or lumpiness at the gill base that might indicate a goiterous condition. While pathological evaluation of the tissues is needed for a definitive determination of the state of the thyroid, there were no easily recognizable growths present in any of the fish sampled. Informal discussions with both sport and commercial fishermen operating throughout Lake

Michigan confirmed that a noticable growth at the gill base is a fairly rare phenomenon. The lack of readily observable goiters in the fish is probably a function of several factors, including the average age of the animal, residence time in contaminated areas, etc. However, the important point as regards this study is that the presence of a goiter does not seem to be a viable indication of low-level contamination of the sports-fishing species.

The levels of free and total tri-iodothyronine and thyroxine were determined for each sample by radioimmunological assay. The average values, standard deviation and range for each parameter, species and sex are presented in Table I.

Serum levels of the active form of vitamin A, retinol, were determined by HPLC analysis of an extract of serum. Dioxin, and similarly acting compounds have been demonstrated to eventually cause pronounced depression of levels of vitamin A in the serum of treated animals. The values determined for this parameter are also presented in Table I.

The rather large variation in the hormone and vitamin A levels observed in the samples is probably primarily due to age and seasonal variations within the sample group.

A relative indication of contamination of the fish by polyhalogenated aromatic hydrocarbons (PAH) was made by gas chromatographic analysis of a de-lipidized extract of the sample liver. Samples were analysed by conditions utilized for a broad spectrum of PAH's, and the total peak area observed in the specific region of the chromatogram corresponding to the retention time of a majority of PAH's was determined. This total peak area was expressed in terms of a DDT standard analyzed by this same method. The average contamination of the samples appears to be greater in the lake trout, a longer-lived species, and one thought to be routinely contaminated to a greater extent than either of the

other species studied. The average liver contamination levels are presented in Table II.

For the evaluation of the contamination of the fish by a mouse bioassay, two lake trout and two king salmon samples were selected, on the basis of total T_3 and total T_4 as being contaminated at a high or low level, one per species. Extracts prepared from the fish livers were injected intraperitoneally into male Balb C/57 mice, a "responder species" with respect to induction of the so called "Ah locus". Ah locus induction is a symptomatic characteristic of the activity of the class of compounds of which dioxin is the most potent member. These compounds also characteristicly cause proliferation of endoplasmic reticulum, and an increase in the total content of cytochrome P448. Microsomes were prepared from the fish liver extract, and control treated mice livers, and the concentrations of cytochrome P448 measured by the carbon monoxide difference spectra of reduced P448. Ah locus induction was determined by a measurement of microsomal Aryl Hydrocarbon Hydroxylase, as Ethoxyresorufin-O-deethylase (EROD) activity. The sample number, thyroid hormone levels, P448 content and EROD activity are presented in Table III.

We did not observe a statistically significant correlation between any of the thyroid hormone or serum retinol levels, and the relative contamination of the sample as measured by gas chromotographic analysis. Further, we were unable to generate Ah locus and P448 induction in mice by injections of liver extracts. The data do not therefore, appear to support the hypothesis that contamination of the fish by dioxin type compounds inversely correlates with levels of thyroid hormones in the serum.

Table 1 - Average Values, Standard Deviation and Range for Serum Thyroid Hormone and Retinol Levels

Species Sex (n)	$\bar{x}$, S.D. and Range				
	FT3[1]	TT3[2]	FT4[3]	TT4[4]	Retinol[5]
King Salmon					
Male (18)	42.3, 29.0	20.1, 9.5	1.38, 1.04	14.9, 6.7	0.73, 0.41
	11.1-118.0	2.4- 39.0	0.37- 3.50	6.3- 27.8	0.20- 1.38
Female (20)	49.5, 35.0	23.1, 10.6	1.38, 0.93	15.0, 6.6	0.85, 0.40
	10.8-163.0	6.5- 53.9	0.62- 4.60	7.9- 35.1	0.33- 1.48
Coho Salmon					
Male (4)	47.1, 18.0	22.9, 6.8	1.50, 0.80	21.5, 3.3	0.87, 0.39
	22.5- 67.6	14.4- 32.3	0.97- 2.83	16.1- 24.4	0.40- 1.33
Female (3)	33.5, 17.0	24.0, 6.2	1.03, 0.41	17.0, 4.3	0.66, 0.25
	15.4- 57.3	15.8- 30.8	0.80- 1.61	12.2- 22.7	0.33- 0.85
Lake Trout					
Male (6)	39.4, 13.0	10.1, 6.1	0.58, 0.14	7.4, 1.4	0.56, 0.22
	23.8- 67.6	6.0- 22.8	0.37- 0.74	4.8- 9.5	0.28- 1.00
Female (6)	22.1, 8.9	8.0, 4.5	0.65, 0.24	8.1, 2.8	0.57, 0.27
	8.6- 31.8	3.3- 15.0	0.40- 1.00	4.6- 13.5	0.18- 1.00

[1]FT3 = Free T_3, pmol/dl
[2]TT3 = Total T_3, nmol/l
[3]FT4 = Free T_4, pmol/l
[4]TT4 = Total T_4, nmol/l
[5]Retinol, µg/ml

Table II - Contamination Levels in Fish Liver Sample Aliquot

Species (n)	DDT-PPM Equivalent
King Salmon (35)	20.6 1.34-70.4
Coho Samon (7)	21.5 3.68-61.6
Lake Trout (10)	40.8 6.56-130

Table III - Results of Mouse Bioassay

Fish Sample	Salmon Serum TT4 (nmol)	Salmon Serum TT3 (nmol)	Salmon Liver Extract DDT-PPM Equivalent	Mouse Liver Microsomal [Cyt P448] nmol/mg.protein	Mouse Liver Microsomal EROD nmol/mg.protein
6 - 27 - 9 ♂ L.T.	6.9	3.3	82	.565	.541
6 - 27 - 12 ♀ L.T.	7.3	15.0	13	1.180	.650
8 - 17 - 1 ♂ King	4.9	2.4	70	1.150	.520
8 - 18 - 12 ♀ King	23.5	35.4	46	1.310	.676
Control O:1 (Peanut)	----	----	----	1.550	.670
Control O:1 (Peanut)	----	----	----	1.010	.632

2. **Titles of Manuscripts and Dissertations Arising from the Work:**

   None

3. **Names and Titles of Personnel Engaged in the Project:**

   Robert Powers, Graduate Research Assistant

   Anne Marie Smykay, Undergraduate Research Assistant

4. **Copies of Published Manuscripts:**

   None

5. **Description and Status of Inventions:**

   None

6. **Signature of Principal Investigator and Date:**

Steven D. Aust — 10/22/85

Steven D. Aust — Date